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Potentiated reinstatement of cocaine-seeking behavior following amphetamine infusion into the basolateral amygdala

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ABSTRACT

Rationale: Reinstatement of extinguished drug-seeking behavior following chronic drug self-administration has been demonstrated in rats in the presence of conditioned cues. We have previously shown that blockade of dopamine (DA) D1 receptors in the basolateral amygdala (BLA) abolishes conditioned cue-induced reinstatement of cocaine-seeking behavior. *Objective:* The present study tested the hypothesis that *d*-amphetamine-induced facilitation of monoamine neurotransmission in the BLA would facilitate conditioned cue-induced reinstatement of extinguished drug-seeking behavior. *Methods:* During daily self-administration sessions over 10 consecutive days, rats pressed a lever to receive cocaine infusions (0.2 mg/0.05 ml) paired with a tone+light compound stimulus. Following self-administration, rats underwent daily extinction sessions, during which no stimuli were presented. On the test days, rats received intra-BLA *d*-amphetamine (10 or 30 μg/side) or vehicle infusions followed by extinction and conditioned cue-induced reinstatement testing. *Results: D*-amphetamine infusions did not alter extinction responding relative to vehicle infusions. During reinstatement testing, conditioned-cue presentation significantly increased responding over extinction levels, and intra-BLA *d*-amphetamine produced a dose-dependent increase in responding relative to vehicle infusions. *Conclusion:* These findings suggest that enhanced monoamine tone in the BLA potentiates the motivational effect and/or salience of cocaine-paired cues during reinstatement.

Key words: *d*-amphetamine, basolateral amygdala, cocaine, dopamine, reinstatement

Relapse to drug-seeking and drug-taking presents a major challenge to the treatment of addiction to drugs of abuse, such as cocaine. Among the variables that contribute to drug relapse, exposure to drug-associated environmental cues is a very powerful factor (O'Brien et al. 1990; Childress et al. 1993). Cue-induced relapse occurs when initially neutral stimuli (e.g. paraphernalia or environment) are repeatedly paired with the effects of the drug, thus acquiring conditioned motivational properties over time (Stewart et al. 1984). In cocaine users, exposure to stimuli previously paired with drug-taking behavior can elicit craving and conditioned physiological arousal (Childress et al. 1988; O'Brien et al. 1990; O'Brien et al. 1992; Childress et al. 1993).

Similar to humans, environmental stimuli (e.g., lights or tones) consistently paired with drugs of abuse elicit reinstatement of extinguished drug-seeking behavior (e.g., non-reinforced lever presses) in laboratory animals (Davis and Smith 1976; de Wit and Stewart 1981; Meil and See 1996; See et al. 1999). The ability of drug-paired stimuli to elicit drug-seeking behavior in animals is reliable, robust, and persistent over time. One study has even demonstrated reinstatement in response to a drug predictive stimulus four months after cessation of cocaine taking (Ciccocioppo et al. 2001). The condition-cued reinstatement model thus possesses strong predictive and face validity in relation to cue-induced relapse in humans (Markou et al. 1993; Carroll and Comer 1996; Fuchs et al. 1998). In this model, rats are trained to lever press for intravenous cocaine infusions paired with a compound light + tone stimulus during daily self-administration sessions. The self-administration days are followed by extinction days, during which lever responding declines and extinguishes in the absence of drug reinforcement. On the test day, drug-seeking behavior is measured in the presence of the compound stimulus presented

response contingently. It is assumed that drug-seeking behavior is an index of incentive motivation for cocaine.

A structure that has been implicated in reinstatement of drug-seeking behavior is the basolateral amygdala (BLA), which is composed of the lateral and basolateral nuclei of the amygdala. Evidence suggests that the BLA is critical for stimulus-reward associations. For instance, lesions of the BLA do not disrupt the ability of primary reinforcers (Whitelaw et al. 1996; Meil and See 1997), but impair the ability of secondary reinforcers (Cador et al. 1989; Everitt et al. 1989), to control goal-directed behavior. BLA lesions suppress second-order responding for conditioned reinforcers (CR) associated with sexual reinforcement (Everitt et al. 1989) or water reward (Cador et al. 1989). Lesions of the BLA also impair appetitive Pavlovian conditioning and first-order conditioned-reinforcers following post-conditioning devaluation of a food US (Hatfield et al. 1996). Lesions of the BLA severely impair acquisition of cocaine self-administration under second order schedule reinforcement, thus indicating the importance of this limbic structure in mediating conditioned reinforcing effects of drug-paired cues (Whitelaw et al. 1996). Temporary inactivation of the BLA by tetrodotoxin (TTX) during classical-conditioning sessions where cocaine infusions were paired with light and tone disrupted acquisition and expression of cue-reinstatement behavior (Kruzich and See 2001). Furthermore, Fos protein expression is increased in the BLA in response to exposure to cocaine-associated stimuli (Neisewander et al. 2000). Similarly, the amygdala becomes highly activated upon exposure to drug-paired cues in the brains of human cocaine users (Grant et al. 1996; Childress et al. 1999). Taken together, these findings demonstrate the importance of the BLA in stimulus-reward associations.

Dopamine (DA) neuronal terminals are densely localized in the BLA (Fallon et al. 1978; Brinley-Reed and McDonald 1999). Only when animals self-administer cocaine, as opposed to receiving passive cocaine infusions, are DA levels increased in the amygdala, suggesting that increases in DA neurotransmission in the amygdala is linked to drug-taking and perhaps drug-seeking behavior (Wilson et al. 1994). Consistent with the idea that BLA DA is important in cue-induced cocaine-seeking behavior, intra-BLA infusion of SCH 23390, D1 receptor antagonist, abolished cue-induced reinstatement (See et al. 2001). In the present study, *d*-amphetamine was infused into the BLA to increase extracellular DA locally, in order to further investigate the role of DA in the BLA. Intra-BLA *d*-amphetamine was hypothesized to enhance cocaine-seeking behavior in the presence of cocaine-associated cues.

MATERIALS AND METHODS

Animals

Male Sprague-Dawley rats (275-350 g at the time of surgery) were individually housed and maintained on a 12-hour reverse light/dark cycle (lights on at 1800). All protocols were approved by an Institutional Animal Care and Use Committee, and were conducted in accordance with the "Principles of laboratory animal care" (NIH publication No. 86-23, revised 1985).

Food Training

Rats were food restricted to approximately 90% of their ad libitum weight for 2 days prior to food training and trained to lever press for 45-mg food pellets (formula A/I; Research Diets, Inc., New Brunswick, NJ). The training occurred on a fixed ratio 1 (FR1) schedule during

an overnight session (16-h), during which no stimuli were presented. Subjects that did not meet a minimum criterion of >100 reinforced responses/session were given an additional training session. After food training, the food hoppers were removed from the chambers and replaced with metal panels. Rats were maintained on 20-30 g of rodent chow per day for the duration of the study.

Surgery

One day after the completion of food training, rats were anesthetized with intraperitoneal ketamine (64.68 mg/kg), xylazine (1.26 mg/kg) and Equithesin (1 ml/kg) prior to surgical implantation of indwelling i.v. catheters. Catheters were constructed from Silastic laboratory grade tubing (0.64 mm i.d., 1.19 mm o.d.; Dow Corning, Midland, MI) attached to a 22-gauge cannula (Plastics One Inc., Roanoke, VA). The catheter/cannula assembly was affixed to polypropylene mesh (Small Parts, Miami Lakes, FL) with cranioplastic cement. A hardened silicon gel (Silicone sealant; General Electric Sealants and Adhesives, Huntersville, NC) nodule was affixed to the catheter 33 mm from the tip of the catheter. The catheter was implanted into the right jugular vein and secured in place with silk suture around the silicon nodule. The Silastic tubing ran under the skin to an exit point in the mid-scapular region, where the opening of the threaded cannula was covered with a threaded plastic cap when not in use. Following catheter implantation, rats were mounted into a stereotaxic apparatus and 26-gauge stainless steel guide cannulae were bilaterally aimed 2 mm above the BLA (A/P= -2.7; L= +/- 5.0; V= -6.6) relative to bregma (Paxinos and Watson 1986). The guide cannulae were held in place with cranioplastic cement anchored to the skull with three steel screws. Stainless steel stylets (Plastics One Inc.) were inserted into the guide cannulae following surgery. Rats were infused i.v. with 0.1 ml each of cefazolin (100 mg/ml) and heparinized saline (70 U/ml) twice daily

during a 4-day recovery period. Catheter patency was verified by infusing 0.1 ml of methohexital sodium (10 mg/ml, IV; Eli Lilly and Co., Indianapolis, IN), which produces temporary loss of muscle tone only when administered intravenously.

Self-Administration

Self-administration training was conducted in standard operant conditioning chambers (30 X 20 X 24 cm; Med Associates, St. Albans VT). Each chamber contained two levers (7 cm above the chamber floor) on either side of a food pellet receptacle (the food receptacle was present only during food training). A white circular stimulus light (2.5 W, 24-V bulb) was located 7 cm above the active lever, and a red house light (2.5W, 24-V bulb) was located on the wall on the opposite end of the chamber. The infusion line was attached to a liquid swivel (Instech, Plymouth Meeting, PA) mounted on a suspended counterbalance. The self-administration apparatus was enclosed in a sound attenuating chamber (Med Associates). Cocaine hydrochloride (4 mg/ml; National Institute on Drug Abuse, Research Triangle Park, NC) was delivered using a computer controlled infusion pump located outside of the sound attenuating chamber. The entire system was computer integrated using Schedule Manager for Windows (version 2.09; Med Associates).

Rats self-administered cocaine on 10 consecutive days during 2-h sessions. Rats received 0.1 ml of heparinized saline (10 U/ml, i.v.) prior to each self-administration session. The animals were then connected to the drug infusion line and the session was initiated. Each session began with illumination of the red house light that remained lit for the entire session. Responses on the right (active) lever resulted in delivery of a cocaine infusion (0.2 mg/ 0.05 ml bolus) over 2 s. Responses on the left lever had no programmed consequences, but were also recorded. Each infusion was paired with a 5-s compound stimulus presentation, consisting of the white

stimulus light over the right lever and a tone (2 kHz, 78 dB) delivered via a programmable audio generator (ANL-926; Med Associates). Following cocaine delivery and stimulus presentation, responses on the active lever had no programmed consequences (no cocaine or stimulus delivery) for 40 s, but lever responses were recorded. Following each self-administration session, rats were administered 0.1 ml of cefazolin and 0.1 ml of heparinized saline (70 U/ml) i.v., and catheter ports were closed to maintain patency.

Extinction

Following self-administration training, animals experienced daily 2-h extinction sessions on 6 consecutive days. During the extinction sessions, responding on either lever had no programmed consequences. In order to acclimatize the animals to the intracranial infusion procedure, all animals were given a sham infusion just prior to the fifth extinction session. The sham infusion procedure consisted of inserting the infusion cannulae into the guide cannulae and gently restraining the animal for 4 min. The infusion cannulae (33-gauge) projected 2 mm below the tip of the guide cannulae.

Testing

Prior to testing, the animals had to meet an extinction criterion of 20 or fewer responses on the active lever on at least two consecutive days. Test sessions were divided into extinction tests (lever presses did not elicit cue presentation) and reinstatement tests (light+tone cues were presented upon active lever responses). Prior to each test, the animals were bilaterally infused with either *d*-amphetamine sulfate (10 μg/0.5μl/side, n=15 or 30 μg/0.5μl/side, n=18) or phosphate buffered saline (PBS) vehicle (0.5 μl/side, n=7) into the BLA. These doses were selected based on previous studies that used intracranial *d*-amphetamine to elicit behavioral potentiation in conditioned reinforcement paradigms (Taylor and Robbins 1984; 1986; Cador et

al. 1991). All animals received each test session in a counterbalanced order. An additional control group received vehicle infusions on every test day. Immediately prior to a test session, infusion cannulae (33-gauge) were bilaterally inserted into the guide cannulae such that the tip of the injector extended 2 mm below the tip of the guide cannulae. The injection cannulae were connected via Tygon tubing (0.02 in i.d., 0.06 in o.d.; Norton Performance Plastics, Akron, OH) to gastight syringes (10 μL; Hamilton, Reno, NV). The syringes were set in a PHD 2000 infusion pump (Harvard Apparatus, Holliston, MA). Infusions were delivered at a volume of 0.5 μl over 2 min. The infusion cannulae were left in place for 1 min before and after infusions. After the infusion, cannulae were removed, stylets were inserted, and the animals were placed into the chamber for testing. Prior to each test session, rats received additional extinction days until they reached the extinction criterion.

Locomotor Activity Testing

Effects of intra-BLA *d*-amphetamine on general motor activity were assessed 48 h after the last test session. The animals were infused with *d*-amphetamine (10 μg/side or 30 μg/side) or PBS vehicle. Immediately after the infusion, the animals were placed in an open field photo beam activity system (Plexiglas enclosure 40.8 cm W x 40.8 cm L x 37.5 cm H). Two arrays of 16 photo beam detectors and emitters were spaced along each horizontal side 8 cm apart 4.5 cm above floor and also 2.5 cm apart 15 cm above chamber floor (San Diego Instruments, Inc., San Diego, CA). The total number of photo beam breaks was recorded for 1 h by a computer program.

Histology

After the last test session, rats received an overdose of Equithesin. Rats were then perfused with phosphate buffered saline and 10% formaldehyde. Brains were then extracted and

stored in 10% formaldehyde. Coronal sections (75 μm) were cut using a vibratome, mounted onto gelatin-coated slides, and then stained with cresyl violet. Slides were examined to determine the most ventral point of the infusion cannula tracts. Data of subjects with misplaced cannulae were omitted from data analysis and those subjects are not reported in the numbers above.

Data Analysis

T-tests were used to check for test day order effect between groups. Analyses of variance (ANOVA) were used to analyze responses on the active and inactive levers with lever (active, inactive), day, test day (amphetamine test day, vehicle test day), and cue presentation (cue, no cue) as within subjects factors and group (0, 10, 30 μg/side of *d*-amphetamine) as a between subjects factor, where appropriate. Interaction effects were further investigated using simple main effect tests (t-test) or Tukey HSD post-hoc tests, where appropriate.

RESULTS

Histology

A schematic illustrating cannula placements in the brains of rats that received 0, 10 or 30 μg/side of *d*-amphetamine is shown in Figure 1. Guide cannula tracts were observed in the parietal cortex, caudate-putamen, and in the lateral/basolateral amygdaloid complex.

Self-administration

Rats in each group exhibited stable responding during the last 3 self-administration sessions, with a within-subject variability of less than 10% in daily cocaine intake. There was no difference in cocaine intake between the groups. Collapsed across groups, the mean number of

daily cocaine infusions (±SEM) was 29.51±0.93 (approximately 18.60±0.53 mg/kg per 2-h session). A 2x3x3 mixed factor ANOVA of lever presses on the last three days of self-administration revealed only a significant lever main effect [$F(1,37)=258.04$, $P<.0001$], but no lever by day by group [$F(4,74)=1.40$, $P>.05$], lever by group [$F(2,37)=.02$, $P>.05$], day by group [$F(4,74)=.90$, $P>.05$], or lever by day interaction effects [$F(2,74)=.00$, $P>.05$]. There were also no day [$F(2,74)=.28$, $P>.05$] or group main effects [$F(2,37)=.23$, $P>.05$]. Thus, animals responded more on the active lever than on the inactive lever, regardless of day, and the three groups did not differ in cocaine self-administration history.

Extinction

Upon removal of cocaine reinforcement, active lever responding for all groups decreased over the course of daily extinction sessions (Figure 2). A 2x2x3 mixed factor ANOVA of lever presses revealed a significant day by lever interaction effect [$F(1,37)=202.01$, $P<.0001$], significant day [$F(1,37)=61.77$, $P<.0001$] and lever main effects [$F(1,37)=271.72$, $P<.0001$], but failed to show a day by lever by group [$F(2,37)=.30$, $P>.05$], day by group [$F(2,37)=.08$, $P>.05$], or lever by group interaction effect [$F(2,37)=.18$, $P>.05$], or a group main effect [$F(2,37)=.38$, $P>.05$]. Subsequent simple main effect tests revealed a significant decrease in active lever responding [$t(39)=12.46$, $P<.0001$] and an increase in inactive lever responding [$t(39)= -9.94$, $P<.001$] during extinction relative to self-administration. Furthermore, the analyses showed significantly lower number of responses on the inactive lever relative to active lever during both self-administration [$t(39)= -17.84$, $P<.0001$] and extinction [$t(39)= -5.68$, $P<.0001$].

Reinstatement testing

Tests for order effects of treatment revealed no significant effects; thus, data were collapsed across test day order. Furthermore, there was no residual effect of amphetamine

administration on active lever responding on subsequent vehicle test days. A mixed factor ANOVA revealed that there was a significant cue presentation main effect [$F(1,19)=21.31$, $P<.0001$], but there was no group by cue presentation interaction effect [$F(2,19)=.84$, $P>.05$], nor was there a group main effect [$F(2,19)=3.26$, $P>.05$]. Thus, there was no difference in active lever responding between groups that received vehicle after a *d*-amphetamine infusion or after a vehicle infusion (e.g., vehicle only control group).

In the absence of cues, intra-BLA *d*-amphetamine failed to alter responding on the active and inactive levers. A 2x2 mixed factor ANOVA of active lever presses in the absence of cue presentation failed to reveal a test day by group interaction effect [$F(1,31)=.00$, $P>.05$], and test day [$F(1,31)=2.80$, $P>.05$] or group main effects [$F(1,31)=.04$, $P>.05$]. A similar analysis of inactive lever presses in the absence of cues failed to reveal a test day by group interaction effect [$F(1,31)=.82$, $P>.05$], and test day [$F(1,31)=.40$, $P>.05$] or group main effects [$F(1,31)=.02$, $P>.05$]. Thus, in the absence of cues, there was no difference in responding on the active and inactive levers following 10 or 30 μg/side of *d*-amphetamine infusion into the BLA relative to vehicle infusion.

In contrast to responding under extinction conditions, rats responded significantly more following intra-BLA *d*-amphetamine infusion than following a vehicle infusion in the presence of conditioned cues. A 2x2 mixed factor ANOVA of active lever presses in the presence of response-contingent cues revealed significant test day [$F(1,31)=10.41$, $P<.05$] and group main effects [$F(1,31)=6.10$, $P<.05$], but no test day by group interaction effect [$F(1,31)=1.29$, $P>.05$]. Furthermore, a planned comparison indicated that the 30 μg/side dose group responded significantly more than the 10 μg/side dose group following *d*-amphetamine infusion [$t(31)$, $P<.05$], but not following vehicle infusion [$t(31)$, $P>.05$]. Potentiation of lever responding was

limited to the previously drug-paired lever, in that analysis of inactive lever presses in the presence of response-contingent cues failed to reveal a test day by group interaction effect [$F(1,31)=.20$, $P>.05$], and a test day [$F(1,31)=.46$, $P>.05$] or group main effect [$F(1,31)=.09$, $P>.05$].

Temporal analysis of active lever responding

Further analysis of lever responding was conducted in order to ascertain the temporal pattern of *d*-amphetamine-induced potentiation of responding. *D*-amphetamine dose-dependently increased active lever responding during the first 30-min bin (Figure 3). A 2x3x4 repeated measures ANOVA of lever responses revealed a significant three-way group by test phase by bin interaction effect [$F(6,174)=2.20$, $P<.05$], bin by group [$F(3,87)=7.20$, $P<.0001$] and test day by bin interaction effects [$F(6,174)=4.40$, $P<.0001$], and test day [$F(2,58)=5.81$, $P<.01$], bin [$F(3,87)=23.32$, $P<.0001$], and group main effects [$F(1,29)=6.51$, $P<.05$]. There was no day by group interaction effect [$F(2,58)=1.88$, $P>.05$]. Subsequent pairwise comparisons indicated that 10 µg/side of *d*-amphetamine did not increase responding relative to either the last day of self-administration (Tukey HSD, $P>.05$) or the vehicle reinstatement test day (Tukey HSD, $P>.05$). In contrast, the 30 µg/side of *d*-amphetamine significantly increased lever responding in the presence of cues during the first 30-min bin, relative to the last day of self-administration (Tukey HSD, $P<.05$) or to the vehicle reinstatement day (Tukey HSD, $P<.05$). This potentiation was not apparent following the first 30-min bin.

Locomotor Testing

Intra-BLA *d*-amphetamine produced a modest increase in general motor activity as measured by the total number of beam breaks during the 1-h locomotor activity test (Figure 4). A 2x3 mixed factor ANOVA of locomotor activity during 30-min bins revealed significant bin

[F(1,33)=203.02, P<.0001] and group main effects [F(1,33)=6.49, P<.01], but failed to show a bin by group interaction effect [F(2,33)=.64, P>.05]. Collapsed across bins, both 10 (Tukey HSD, P<.05) and 30 μg/side of *d*-amphetamine (Tukey HSD, P<.05) significantly increased locomotor activity relative to vehicle.

DISCUSSION

The present study examined the effects of *d*-amphetamine infusion into the basolateral amygdala (BLA) on the expression of condition-cued reinstatement of cocaine-seeking behavior. Intra-BLA *d*-amphetamine infusions dose-dependently increased condition-cued reinstatement of extinguished cocaine-seeking behavior without altering responding under extinction conditions (Figure 2). Potentiation of condition-cued reinstatement of cocaine-seeking behavior elicited by the 30 μg/side dose of *d*-amphetamine primarily occurred during the first 30 minutes of testing (Figure 3).

Previous research suggests that the BLA is important in the utilization of stimulus-reward associations (Whitelaw et al. 1996). Post-training lesions of the BLA inhibit expression of discriminative approach to a CS previously paired with sucrose (Burns et al. 1993). Post-training BLA lesions abolish conditioned cue-induced cocaine-seeking behavior (Meil and See 1997). Furthermore, temporary inactivation of the BLA by lidocaine (Kantak et al. 2002) or TTX (Grimm and See 2000) impair the ability of drug-paired cues to reinstate drug-seeking behavior. Thus, intact function of the BLA is necessary for the utilization of previously learned stimulus-reward associations. The present study extended our understanding of the role that monoamines in the BLA play in utilizing previously learned stimulus-reward associations.

It is unlikely that *d*-amphetamine reward accounted for potentiation of condition-cued reinstatement because previous research indicates that the BLA does not mediate the rewarding effects of psychomotor stimulants and other primary reinforcers. Consistent with this idea, BLA lesions do not disrupt responding for water or access to an estrous female on a continuous reinforcement schedule (Cador et al. 1989; Everitt et al. 1989). Furthermore, intra-BLA infusion of *d*-amphetamine (5 μg/side) does not elicit conditioned place preference (O'Dell et al. 1999). These findings mitigate the possibility that *d*-amphetamine served as a drug prime in the present study, especially since intra-BLA infusions did not alter responding under extinction conditions.

Previous reports indicate that DA levels in the amygdala become elevated during cocaine self-administration and cocaine-seeking behavior (Tran-Nguyen et al. 1998; Weiss et al. 2000). Specifically, during cocaine self-administration, DA levels increase in the amygdala and they are significantly higher than DA levels in animals that received passive infusions of cocaine (Wilson et al. 1994). Furthermore, DA levels increase in the amygdala following chronic cocaine intake during cocaine-seeking behavior responding in a cocaine-paired environment (Tran-Nguyen et al. 1998) and during reinstatement of extinguished cocaine-seeking behavior elicited by presentation of a cocaine predictive CS+ (Weiss et al. 2000) or a cocaine priming injection (Tran-Nguyen et al. 1998). Similarly, a CS+ paired with sucrose elicits an increase in DA overflow in the amygdala, while a CS- does not elicit the same DA increase (Harmer and Phillips 1999). Collectively, these findings suggest that motivationally relevant cues influence DA levels in the amygdala and that DA in the amygdala may play a role in stimulus-reward associations and subsequent goal-directed behavior.

D1 DA receptors in the BLA play a critical role in condition-cued reinstatement of extinguished cocaine-seeking behavior. Systemically administered D1 antagonists, SCH 39166

or SCH 23390, reduce cocaine-seeking behavior and Fos expression in the BLA and medial prefrontal cortex in the presence of a cocaine-predictive stimulus (Ciccocioppo et al. 2001). Additionally, intra-BLA infusion of SCH-23390, a D1 receptor antagonist, abolishes expression of condition-cued reinstatement of cocaine-seeking behavior (See et al. 2001). In contrast, intra-BLA infusion of raclopride, a selective D2 antagonist, fails to alter cocaine-seeking behavior (See et al. 2001). These findings demonstrate that stimulation of D1 receptors is necessary for condition-cued reinstatement of cocaine-seeking behavior.

While cocaine-paired stimuli may increase DA levels in the amygdala, that increase may in turn further increase processing of the sensory aspects of these stimuli, thereby facilitating their motivational salience. Electrophysiological studies suggest that DA in the BLA is a powerful modulator of the excitability of interneurons and projection neurons (Rosenkranz and Grace 1999). DA receptor activation, upon application of DA into the BLA, appears to act as a "noise filter" by inhibiting projection neurons and exciting interneurons via the stimulation of D1 receptor. The modulatory effects of DA, however, appear to be selective to the type of input received by the BLA. Specifically, sensory input appears to be potentiated by DA, while limbic input is attenuated. As a result, it has been hypothesized that in the presence of increased DA neurotransmission in the BLA, only strong inputs can drive amygdala contribution to behavior (Rosenkranz and Grace 1999). Thus, in the present study the cocaine-paired cues, in the presence of enhanced intra-BLA DA, may have been processed as stronger sensory stimuli. As a result, *d*-amphetamine may have increased the salience of the cues and subsequently facilitated focused, cocaine-seeking behavior in response to these cocaine-paired cues via its action as an indirect DA agonist.

Alternatively to increasing the motivational effects of conditioned cues, intra-BLA *d*-amphetamine administration may have increased cocaine-seeking behavior via its generalized stimulant effects, since it elicited hyperactivity in rats relative to vehicle infusions in a novel test environment (Figure 4). These findings are consistent with a previous study that has shown an increase in locomotor activity following intra-BLA administration of *d*-amphetamine (5μg/side; O'Dell et al. 1999). However, in the present study, the stimulant effects of *d*-amphetamine infusion did not seem to alter the specificity of operant responding, in that intra-BLA *d*-amphetamine did not increase responding on the inactive lever during condition-cued reinstatement testing and responding on the active or inactive levers during extinction, relative to vehicle infusion. Thus, it is unlikely that *d*-amphetamine-induced hyperactivity alone was responsible for the *d*-amphetamine-induced potentiation of condition-cued reinstatement.

In addition to rich DA input, the BLA receives rich serotonin and moderate norepinephrine innervation from the dorsal raphe and locus coeruleus, respectively (Sadikot and Parent 1990; Ma et al. 1991). Norepinephrine in the BLA is implicated in certain forms of associative learning, such as conditioned taste aversion (Borsini and Rolls 1984), while serotonin has also been implicated in cocaine-seeking behavior (Tran-Nguyen et al. 1999; Baker et al. 2001; Tran-Nguyen et al. 2001). Since *d*-amphetamine potentiates the release and prevents the reuptake of catecholamines and serotonin (Moore 1978), future studies will need to examine the contribution of all monoamines to the potentiation of condition-cued reinstatement of cocaine-seeking behavior elicited by intra-BLA *d*-amphetamine.

In conclusion, intra-BLA infusion of *d*-amphetamine potentiated conditioned cue-induced cocaine-seeking behavior in the present study. DA likely plays an important role in this phenomenon by facilitating sensory inputs into the amygdala. Increased understanding of

neurotransmitter activity in the BLA during conditioned-cued relapse will provide insight into developing pharmacological treatments for conditioned craving and relapse to drug use.

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REFERENCES

Baker DA, Tran-Nguyen LT, Fuchs RA, Neisewander JL (2001) Influence of individual differences and chronic fluoxetine treatment on cocaine-seeking behavior in rats. Psychopharmacology 155: 18-26

Borsini F, Rolls ET (1984) Role of noradrenaline and serotonin in the basolateral region of the amygdala in food preferences and learned taste aversions in the rat. Physiology & Behavior 33: 37-43

Brinley-Reed M, McDonald AJ (1999) Evidence that dopaminergic axons provide a dense innervation of specific neuronal subpopulations in the rat basolateral amygdala. Brain Res 850: 127-35.

Burns LH, Robbins TW, Everitt BJ (1993) Differential effects of excitotoxic lesions of the basolateral amygdala, ventral subiculum and medial prefrontal cortex on responding with conditioned reinforcement and locomotor activity potentiated by intra-accumbens infusions of D-amphetamine. Behav Brain Res 55: 167-83.

Cador M, Robbins TW, Everitt BJ (1989) Involvement of the amygdala in stimulus-reward associations: interaction with the ventral striatum. Neuroscience 30: 77-86

Cador M, Taylor JR, Robbins TW (1991) Potentiation of the effects of reward-related stimuli by dopaminergic- dependent mechanisms in the nucleus accumbens. Psychopharmacology 104: 377-85

Carroll ME, Comer SD (1996) Animal Models of Relapse. Experimental and Clinical Psychopharmacology 4: 11-18

Childress AR, Hole AV, Ehrman RN, Robbins SJ, McLellan AT, O'Brien CP (1993) Cue reactivity and cue reactivity interventions in drug dependence. NIDA Res Monogr 137: 73-95

Childress AR, McLellan AT, Ehrman R, O'Brien CP (1988) Classically conditioned responses in opioid and cocaine dependence: a role in relapse? NIDA Res Monogr 84: 25-43

Childress AR, Mozley PD, McElgin W, Fitzgerald J, Reivich M, O'Brien CP (1999) Limbic activation during cue-induced cocaine craving. Am J Psychiatry 156: 11-8

Ciccocioppo R, Sanno PP, Weiss F (2001) Cocaine-predictive stimulus induces drug-seeking behavior and neural activation in limbic brain regions after multiple months of abstinence: reversal by D1 antagonists. Proc Natl Acad Sci U S A 98: 1976-1981

Davis WM, Smith SG (1976) Role of conditioned reinforcers in the initiation, maintenance and extinction of drug-seeking behavior. Pavlov J Biol Sci 11: 222-36.

de Wit H, Stewart J (1981) Reinstatement of cocaine-reinforced responding in the rat. Psychopharmacology 75: 134-43

Everitt BJ, Cador M, Robbins TW (1989) Interactions between the amygdala and ventral striatum in stimulus- reward associations: studies using a second-order schedule of sexual reinforcement. Neuroscience 30: 63-75

Fallon JH, Koziell DA, Moore RY (1978) Catecholamine innervation of the basal forebrain. II. Amygdala, suprarhinal cortex and entorhinal cortex. J Comp Neurol 180: 509-32.

Fuchs RA, Tran-Nguyen LT, Specio SE, Groff RS, Neisewander JL (1998) Predictive validity of the extinction/reinstatement model of drug craving. Psychopharmacology (Berl) 135: 151-60

Grant S, London ED, Newlin DB, Villemagne VL, Liu X, Contoreggi C, Phillips RL, Kimes AS, Margolin A (1996) Activation of memory circuits during cue-elicited cocaine craving. Proc Natl Acad Sci U S A 93: 12040-5

Grimm JW, See RE (2000) Dissociation of primary and secondary reward-relevant limbic nuclei in an animal model of relapse. Neuropsychopharmacology 22: 473-9

Harmer CJ, Phillips GD (1999) Enhanced dopamine efflux in the amygdala by a predictive, but not a non-predictive, stimulus: facilitation by prior repeated D-amphetamine. Neuroscience 90: 119-30

Hatfield T, Han JS, Conley M, Gallagher M, Holland P (1996) Neurotoxic lesions of basolateral, but not central, amygdala interfere with Pavlovian second-order conditioning and reinforcer devaluation effects. J Neurosci 16: 5256-65

Kantak KM, Black Y, Valencia E, Green-Jordan K, Eichenbaum HB (2002) Dissociable effects of lidocaine inactivation of the rostral and caudal basolateral amygdala on the maintenance and reinstatement of cocaine-seeking behavior in rats. Journal of Neuroscience 22: 1126-1136

Kruzich PJ, See R (2001) Differential contributions of the basolateral and central amygdala in the acquisition and expression of conditioned relapse to cocaine-seeking behavior. Journal of Neuroscience 21: 1-5

Ma QP, Yin GF, Ai MK, Han JS (1991) Serotonergic projections from teh nucleus raphe dorsalis to the amygdala in the rat. Neuroscience Letters 134: 21-24

Markou A, Weiss F, Gold LH, Caine SB, Schulteis G, Koob GF (1993) Animal models of drug craving. Psychopharmacology 112: 163-82

Meil WM, See RE (1996) Conditioned cued responding following prolonged withdrawal from self-administered cocaine in rats: an animal model of relapse. Behav Pharmacol 7: 754-763

Meil WM, See RE (1997) Lesions of the basolateral amygdala abolish the ability of drug associated cues to reinstate responding during withdrawal from self- administered cocaine. Behav Brain Res 87: 139-48

Moore KE (1978) Amphetamines: Biochemical and behavioral actions in animals. In: Iversen LL, Iversen SD, Snyder SH (eds) Handbook of Psychopharmacology. Plenum Press, New York, pp 41-98

Neisewander JL, Baker DA, Fuchs RA, Tran-Nguyen LT, Palmer A, Marshall JF (2000) Fos protein expression and cocaine-seeking behavior in rats after exposure to a cocaine self-administration environment. J Neurosci 20: 798-805

O'Brien C, Childress AR, Ehrman R, Robbins S, McLellan AT (1992) Conditioning mechanisms in drug dependence. Clin Neuropharmacol 15: 66A-67A

O'Brien CP, Childress AR, McLellan T, Ehrman R (1990) Integrating systemic cue exposure with standard treatment in recovering drug dependent patients. Addict Behav 15: 355-65

O'Dell LE, Sussman AN, Meyer KL, Neisewander JL (1999) Behavioral effects of psychomotor stimulant infusions into amygdaloid nuclei. Neuropsychopharmacology 20: 591-602

Paxinos G, Watson D (1986) The Rat Brain in Stereotaxic Coordinates. Academic Press, Academic Press

Rosenkranz JA, Grace AA (1999) Modulation of basolateral amygdala neuronal firing and afferent drive by dopamine receptor activation in vivo. J Neurosci 19: 11027-39

Sadikot AF, Parent A (1990) The monoaminergic innervation of the amygdala in the squirrel monkey: An immunohistochemical study. Neuroscience 36: 431-447

See RE, Grimm JW, Kruzich PJ (2001) Dopamine, but not glutamate, receptor blockade in the basolateral amygdala attenuates conditioned reward in a rat model of relapse to cocaine-seeking behavior. Psychopharmacology 154: 301-310

See RE, Grimm JW, Kruzich PJ, Rustay N (1999) The importance of a compound stimulus in conditioned drug-seeking behavior following one week of extinction from self-administered cocaine in rats. Drug Alcohol Depend 57: 41-9

Stewart J, de Wit H, Eikelboom R (1984) Role of unconditioned and conditioned drug effects in the self- administration of opiates and stimulants. Psychol Rev 91: 251-68.

Taylor JR, Robbins TW (1984) Enhanced behavioural control by conditioned reinforcers following microinjections of d-amphetamine into the nucleus accumbens. Psychopharmacology 84: 405-12

Taylor JR, Robbins TW (1986) 6-Hydroxydopamine lesions of the nucleus accumbens, but not of the caudate nucleus, attenuate enhanced responding with reward-related stimuli produced by intra-accumbens d-amphetamine. Psychopharmacology 90: 390-7

Tran-Nguyen LT, Baker DA, Grote KA, Solano J, Neisewander JL (1999) Serotonin depletion attenuates cocaine-seeking behavior in rats. Psychopharmacology 146: 60-66

Tran-Nguyen LT, Bellew JG, Grote KA, Neisewander JL (2001) Serotonin depletion attenuates cocaine seeking but enhances sucrose seeking and the effects of cocaine priming on reinstatement of cocaine seeking in rats. Psychopharmacology 157: 340-348

Tran-Nguyen LT, Fuchs RA, Coffey GP, Baker DA, O'Dell LE, Neisewander JL (1998) Time-dependent changes in cocaine-seeking behavior and extracellular dopamine levels in the amygdala during cocaine withdrawal. Neuropsychopharmacology 19: 48-59

Weiss F, Maldonado-Vlaar CS, Parsons LH, Kerr TM, Smith DL, Ben-Shahar O (2000) Control of cocaine-seeking behavior by drug-associated stimuli in rats: effects on recovery of extinguished operant-responding and extracellular dopamine levels in amygdala and nucleus accumbens. Proc Natl Acad Sci U S A 97: 4321-6

Whitelaw RB, Markou A, Robbins TW, Everitt BJ (1996) Excitotoxic lesions of the basolateral amygdala impair the acquisition of cocaine-seeking behaviour under a second-order schedule of reinforcement. Psychopharmacology (Berl) 127: 213-24

Wilson JM, Nobrega JN, Corrigall WA, Coen KM, Shannak K, Kish SJ (1994) Amygdala dopamine levels are markedly elevated after self- but not passive-administration of cocaine. Brain Res 668: 39-45

Figure 1. Schematic representation of cannula placement. The X symbols represent the most ventral point of the injection cannula tracks in the brains of animals from the 0, 10, and 30 μg/side amphetamine groups. The numbers to the left of schematic sections represent the approximate distance from bregma.

Figure 2. Responses on the active and inactive levers (mean ± SEM) during self-administration (SA; mean of last 3 days), the last extinction day (EXT), and testing in the absence and presence of the light+tone stimulus complex. Double dagger (‡) represents a significant difference relative to active lever responding during self-administration (ANOVA day simple main effect, $P<.0001$). Asterisk (*) represents a significant difference relative to vehicle infusions (ANOVA test day main effect, $P<.05$). Dagger (†) represents a significant difference relative to 10 μg/side of *d*-amphetamine (planned t-test, $P<.05$).

Figure 3. Time course of active lever responses on the last self-administration day (SA) and on the vehicle and *d*-amphetamine condition-cued reinstatement test days (mean ± SEM). *Top panel*: Effects of intra-BLA infusions of 10 μg/side of *d*-amphetamine. *Bottom panel*: Effects of intra-BLA infusions of 30 μg/side of *d*-amphetamine. Dagger (†) represents significant difference relative to vehicle infusion (Tukey HSD test, $P<.05$). Asterisk(*) represents significant difference relative to self-administration (Tukey HSD test, $P<.05$).

Figure 4. Effects of intra-BLA *d*-amphetamine on motor activity (photo-beam breaks ± SEM). Asterisk (*) represents a significant difference relative to vehicle infusion (Tukey HSD test, $P<.05$).

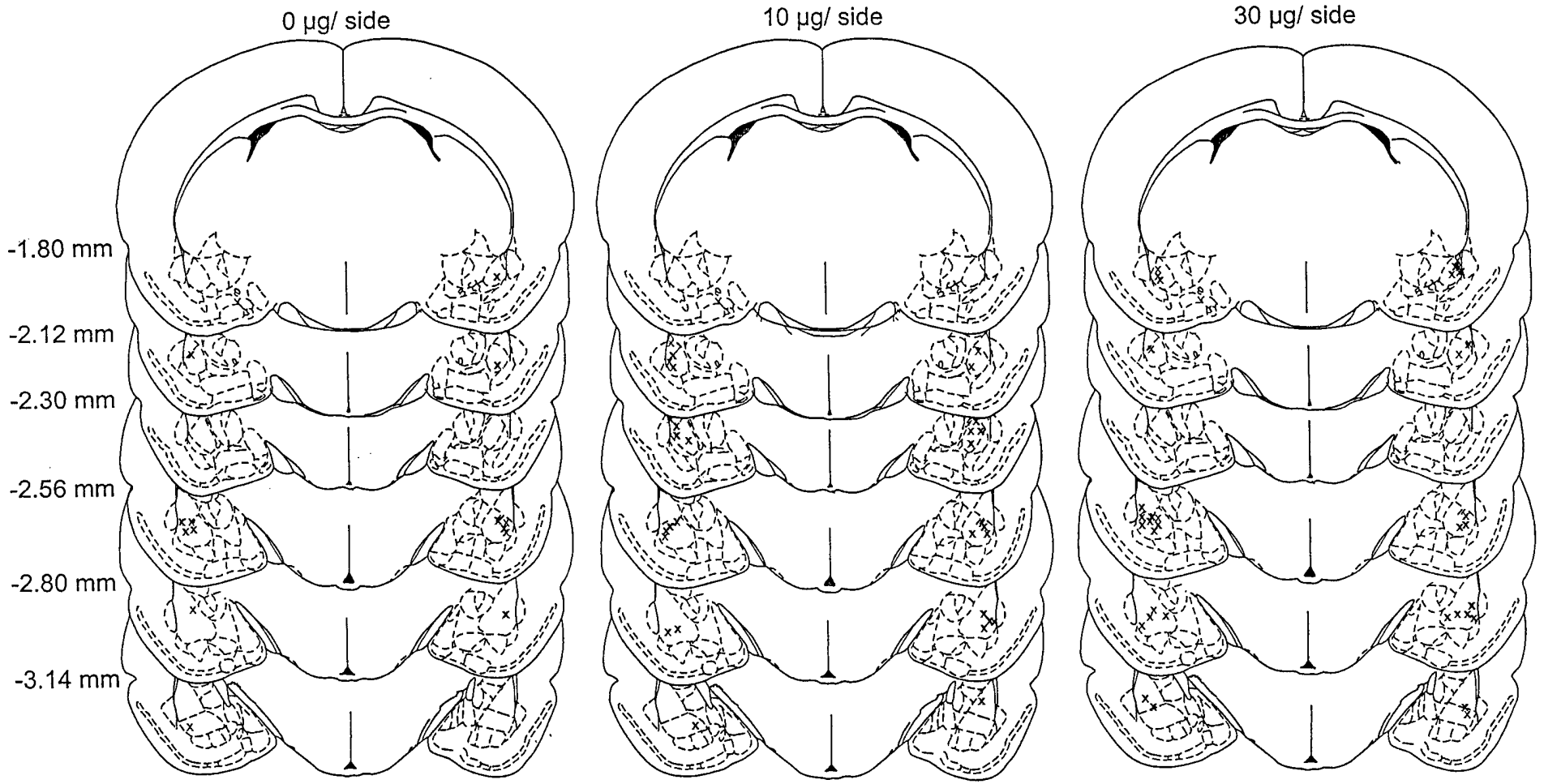
0 µg/ side
10 µg/ side
30 µg/ side
-1.80 mm
-2.12 mm
-2.30 mm
-2.56 mm
-2.80 mm
-3.14 mm

Figure 1

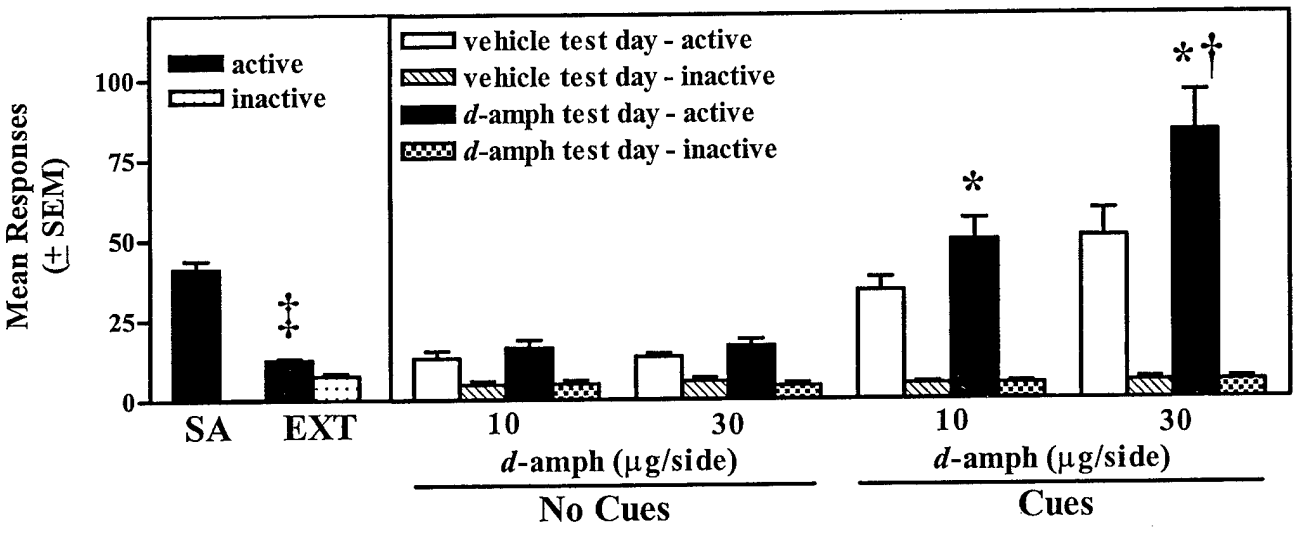
Mean Responses
(± SEM)
100
75
50
25
0
active
inactive
vehicle test day - active
vehicle test day - inactive
d-amph test day - active
d-amph test day - inactive
SA
EXT
10
30
10
30
d-amph (μg/side)
d-amph (μg/side)
No Cues
Cues
Phase of Experiment

Figure 2

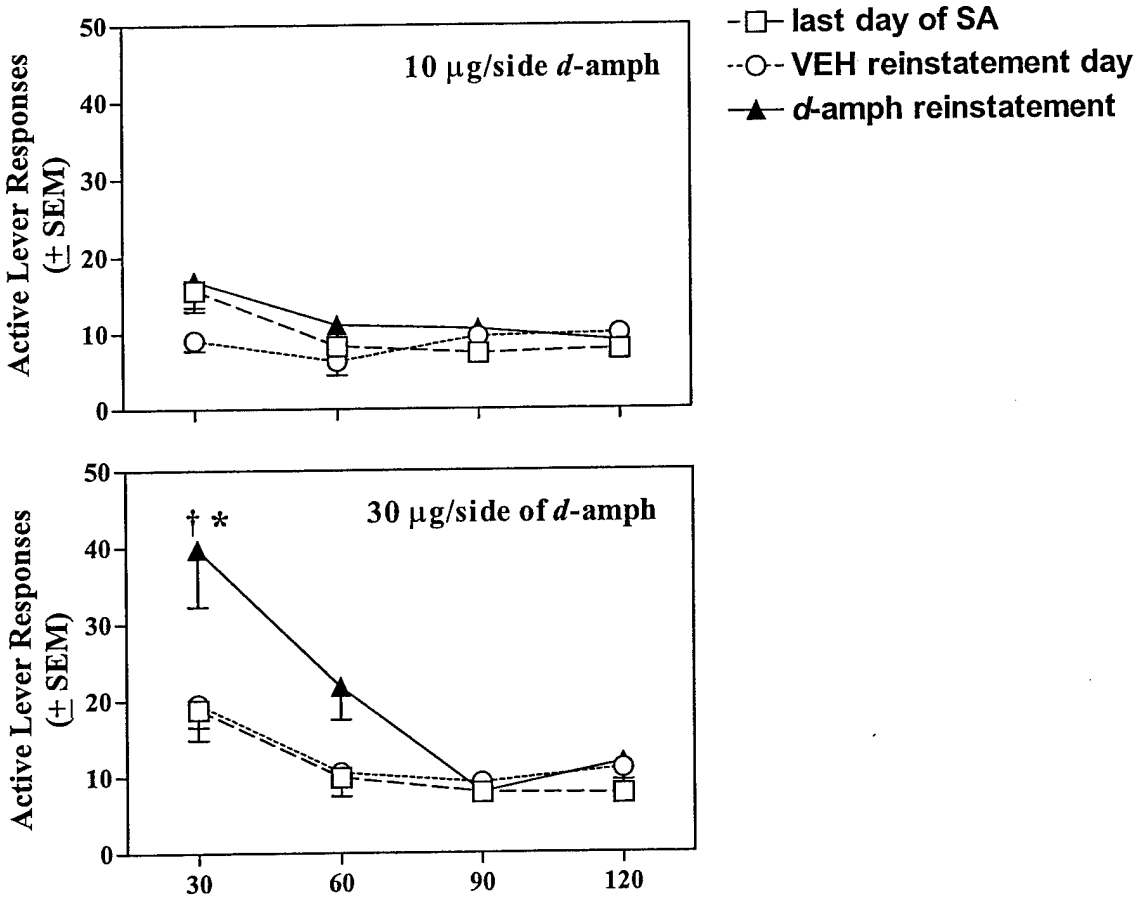
last day of SA
VEH reinstatement day
d-amph reinstatement
10 µg/side d-amph
30 µg/side of d-amph
Active Lever Responses (± SEM)
Active Lever Responses (± SEM)
50
40
30
20
10
0
† *
30
60
90
120

Figure 3

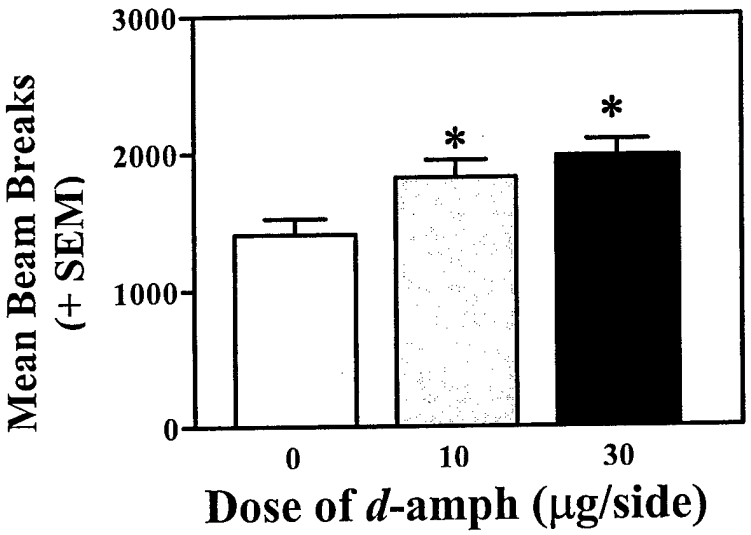
3000
2000
1000
0
Mean Beam Breaks
(+ SEM)
*
*
0
10
30
Dose of d-amph (μg/side)

Figure 4

25 November 2002

To: AFIT/CIGS Program Manager (MaLisa Freeland)

From: Christopher C. Ledford

Subject: PA release form

Attached is a copy of my thesis that may be considered for publication in the future. Please note the Program Manager's endorsement (page 3) still needs to be completed. Please submit this to AFIT/PA for review.

CHRISTOPHER C. LEDFORD, Capt. , USAF
Graduate Student